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# Characterization of the Ah Receptor

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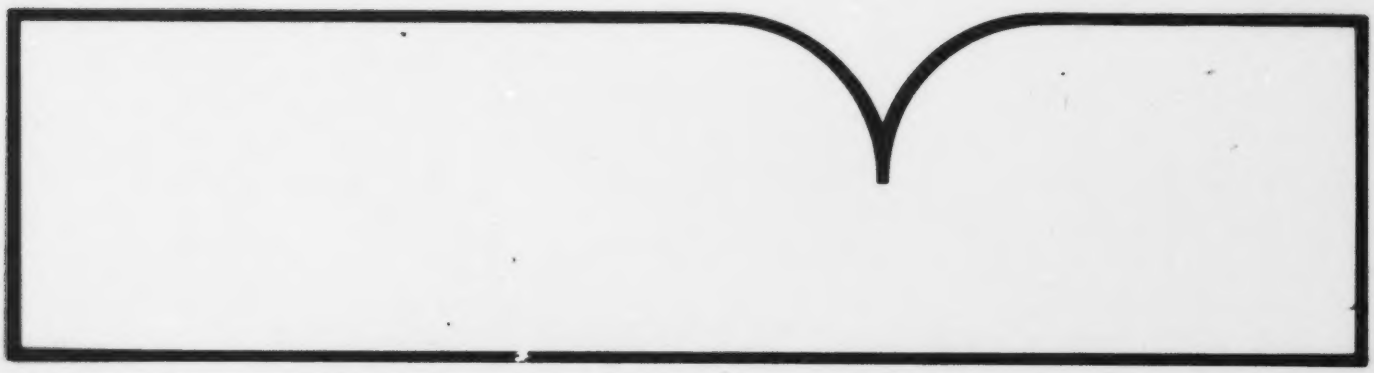

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16. ABSTRACT

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Results are based on a new one-step methodology which allows activation parameters to be calculated directly from raw experimental measurements which allows the uncertainty in the activation enthalpy, expressed as a 95% confidence interval, to be obtained unambiguously.

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# CHARACTERIZATION OF THE Ah RECEPTOR

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ABSTRACT

Receptor degradation occurs competitively with binding over the whole range of temperatures from 4 to 37 Celsius; additionally, there is yet no direct evidence for complex dissociation. Thus, the binding of the ligand stabilizes the receptor to an extent much greater than is found with steroid binding. Two kinetic models are presented. In the first, association is viewed as being in competition with receptor degradation. According to this model, previous estimates of the association constant are orders of magnitude too low, and the initial cytosolic receptor protein concentration has been underestimated by factor of 2-3. In the second, we postulate the slow conversion of the Ah receptor to an activated state which either inactivates or is competitively trapped by 2,3,7,8-TCDD. This presents a novel mechanism of receptor-ligand interaction, namely the activation of the receptor prior to binding.

The rat liver cytosolic receptor protein containing the Ah-receptor protein was purified and studied using a photochemical assembly of 2,3,7,8-TCDD. The receptor protein was purified using various chromatographic procedures. The unbound receptor protein rapidly lost its capacity to bind 2,3,7,8-TCDD. However, the 2,3,7,8-TCDD bound Ah receptor did not readily dissociate, probably reflecting the high potency and persistance of the toxicity of 2,3,7,8-TCDD.

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## INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs) and polybrominated biphenyls (PBBs) are industrial compounds or by-products which elicit a number of common biologic and toxic effects (1-5). Like many hormones, the activities of these toxic halogenated aryl hydrocarbons (HAH) are structure dependent; 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most active HAH and within each class the most toxic members are all approximate isostereomers of 2,3,7,8-TCDD. Many of the effects elicited by this group of toxins are similar to those associated with modulations of several hormone-mediated responses. However, most studies suggest that although toxic HAHs and several classes of hormones can elicit a number of similar biologic and toxic effects, they do so by divergent mechanisms.

Like the steroid hormones, it has been proposed that the effects of the toxic HAHs are dependent on the initial interaction of these compounds with a cytosolic receptor protein (the Ah receptor) in the target tissues (6). Mechanistic studies with 2,3,7,8-TCDD and related halogenated aromatics meet most of the criteria (summarized below) which lend support to the role of the 2,3,7,8-TCDD (or Ah) receptor protein in mediating the pleiotropic responses elicited by this class of environmental toxins.

Evidence for Receptor-Mediated Mechanism

i) Saturable Binding Criteria. The synthesis of radiolabeled 2,3,7,8-TCDD with high specific activity triggered several important mechanistic studies; it was apparent that in the soluble fraction of hepatic and extrahepatic tissues from several species there was a protein which exhibited saturable binding with the radioligand. Moreover, several reports have shown that, 3-methylcholanthrene, benzo[a]pyrene and dibenz[a,h]anthracene also exhibit saturable binding with this cytosolic receptor protein (7).

ii) Tissue or Cellular Specificity. Endogenous receptor ligands such as steroid hormones and neurotransmitters interact with receptors which are located within specific tissues or cells. Tissue specificity has also been demonstrated for the 2,3,7,8-TCDD receptor in rats and mice; C57BL/6J mice and Sprague-Dawley rats which are highly responsive to 2,3,7,8-TCDD exhibit tissue-dependent concentrations of the receptor which vary from 0-54 fmol/mg cytosolic protein. In contrast, non-detectable levels of the receptor protein are observed in cytosol from DBA/2J mice which are relatively non-responsive to the effects of 2,3,7,8-TCDD and related toxic halogenated aryl hydrocarbons.

iii) High Affinity Ligand-Receptor Binding. 2,3,7,8-TCDD, 3-MC and several other toxic halogenated aryl hydrocarbons bind with high affinity to the cytosolic receptor protein with Kd values in the range of 0.1 to 10 nM which approximate Kd values for steroids binding to their cytosolic receptor proteins (7).

iv) Correlation Between Structure-Dependent Binding Specificity and Their Biologic and Toxic Responses. Several studies with PCDD and PCDF congeners clearly demonstrate the effects of structure on their binding affinities, AHH induction potencies and toxicity (reviewed in 1-5). The most active compounds

contain 4 lateral (2,3,7, and 8) Cl substituents and the removal of these groups or the addition of 2 or more non-lateral Cl substituents gives congeners with overall diminished activities. SARS for PBBs and PCBs have shown that the congeners with the highest affinities for the receptor protein are substituted in their lateral positions (4,4',5,5',3 and 3') and these PCBs (3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl) are also the most toxic members of this group of 209 compounds. This coplanar group of PCBs are characterized by their lack of ortho chloro substituents, and they are all approximate isostereomers of 2,3,7,8-TCDD. It has also been demonstrated that a second group of eight PCB congeners, the monoortho-substituted analogs of the coplanar PCBs, exhibit moderate affinities for the cytosolic receptor protein ($IC_{50}$ (binding) = $10^{-5}$ - $10^{-6}$ M) and also elicit the toxic and biologic responses which typify the halogenated aryl hydrocarbons.

v) Genetic Studies. Genetic studies with C57CL/6 and DBA/2 strains of mice and their backcrosses have provided the most cogent evidence in support of the role of the murine Ah receptor in mediating the cellular responses to these toxins. Non-responsive mice contain low levels (< 1 fmol/mg cytosolic protein) of hepatic and extrahepatic Ah receptor protein whereas > 30 fmol/mg cytosolic receptor have been measured in the liver of C57BL/6 mice; these differences in cellular receptor levels are paralleled by the susceptibility of C57BL/6 (high) and DBA/2 (low) mice to the biologic and/or toxic effects of 2,3,7,8-TCDD and related toxic halogenated aryl hydrocarbons. Genetic inbreeding of responsive and non-responsive mice shows that several toxic effects, including teratogenicity, porphyria, immunotoxicity and body weight loss segregate with the Ah locus (8-13).

Although an endogenous ligand has not been identified for the 2,3,7,8-TCDD receptor, it is apparent that this receptor and some hormone receptors initiate several comparable cellular responses. The mode of action of 2,3,7,8-TCDD and related HAHs most closely resembles the mechanism proposed for the steroid hormones and, therefore, studies focused on characterization of the 2,3,7,8-TCDD receptor serve as a useful surrogate for understanding endocrine responses. This proposal will focus on developing methodologies for further characterizing and purifying the 2,3,7,8-TCDD receptor protein.

OBJECTIVES

1. The interaction of several photolabile chemicals with the receptor protein will be determined using a photochemical assembly which has been set up for this study. The first phase of this project will utilize hydrocarbons which exhibit high binding affinities for the Ah receptor and which are available as radiolabeled [$^3$H] compounds with high (20-50 Ci/mmol) specific activity. These compounds include 2,3,7,8-TCDD, 3-methylcholanthrene and benzo[a]pyrene. A second series of photolabile compounds, including azido derivatives of radiolabeled 2,3,7,8-TCDD and benzo[a]pyrene will also be utilized in these photoaffinity studies. The competitive binding of several ligands to the covalently modified receptors will be investigated to probe the possible differences in ligand binding site(s) on the receptor protein(s) and [$^3$H]-photoaffinity labeled-protein adducts will be isolated and used as markers for purification studies.

2. The receptor protein will be purified using a series of chromatographic procedures including ion exchange column chromatography, hydroxyapatite column chromatography affinity chromatography and gel permeation high pressure liquid chromatography. The key step in this approach will be the preparation of

several affinity column supports which have been functionalized with synthetic substituted chlorinated dibenzo-p-dioxins. These functionalized column supports will be utilized to preferentially adsorb the receptor protein from the cytosol and therefore facilitate purification. Since it has been reported that ligand binding activity of the Ah receptor is labile, the purification scheme will utilize the covalently modified radiolabeled ligand-receptor complex as a marker protein.

3. The third major objective of this proposal will focus on the preparation of monoclonal antibodies to the purified Ah receptor. This approach will facilitate the detection and quantitation of this receptor in animal and human tissues and may serve as a probe for determining individual susceptibilities to the biologic and toxic effects elicited by toxic halogenated aryl hydrocarbons.

PROGRESS

1. Initial Ligand Binding Studies

The molecular properties of the crude and partially purified Ah receptor and its interaction with 2,3,7,8-TCDD have been investigated by several groups. Like many of the steroid hormone receptors, the Ah receptor complex is possibly a protein oligomer which contains a hydrophobic binding site and interacts with DNA and polyanions. A recent study has shown that the bound 9S 2,3,7,8-TCDD-receptor protein complex from rat and mouse hepatic cytosol disaggregates in high salt (0.4 M KCl) to give a 4S complex, however, the rate of the 4S-9S complex conversion was more rapid for the rat than the mouse receptor (14). Kester and Gasiewicz (15) have found that the unbound rat hepatic cytosolic receptor is unstable and this can result in an underestimation of cellular receptor concentrations. However, the conventional Scatchard analysis of

2,3,7,8-TCDD receptor binding isotherms assumes that for a simple biomolecular interaction of receptor (R) plus ligand (L) to form a receptor-ligand (RL) complex (equation 1), there is no significant degradation of R, L or RL.

$$R + L \rightleftharpoons RL$$

Beck and Goren (16) have shown that Scatchard plot analyses of biomolecular equilibrium reactions in which any of the components is subject to degradation yield incorrect equilibrium constants and they have proposed kinetic simulation models to re-evaluate such types of data. In our case, where it is the unliganded receptor that is thermally labile, such a kinetic model is shown as Scheme 1.

Scheme 1

$$R + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} RL \qquad (1)$$

$$R \xrightarrow{k_d} \text{Inactivation} \qquad (2)$$

Ideally, such investigations should be carried out with the aid of pure preparations of the Ah receptor, but at the present time this is not possible. The initial studies on understanding receptor-ligand interactions have resulted in the development of two possible kinetic models (see below).

The Scatchard analysis of bimolecular receptor-ligand complexation reactions assumes that the [R], [L] and (RL) values measured at saturation represent the equilibrium concentrations of these species. As shown in Figures 1 and 2 (and as recently observed by Kester and Gasiewicz (15), the unbound receptor rapidly loses its capacity to bind TCDD. Therefore, the concentrations of R, L and RL at saturation will not represent equilibrium concentrations and can lead to inaccurate estimation of $K_{ass}$ and the initial concentration ($R_o$) of receptor

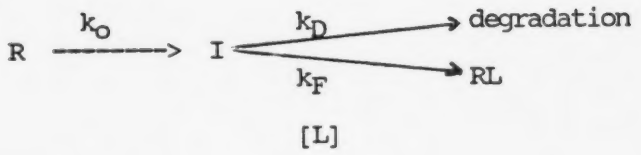
binding sites. We have experimentally determined the values for $k_f$ and $k_d$ and have estimated the values of $k_r$ and $R_o$ by matching the experimental results with the computer stimulated curve (Figure 3). We found that $k_r$ was too small to measure experimentally. The estimated values for $k_r$, $K_{ass}$ and $R_o$ from the simulation curves at 10, 20 and 30°C are summarized in Table 1. Since $k_r$ is a maximum estimate for the dissociation constant, the $K_{ass}$ values are minimum estimates. The $K_{ass}$ value ($3 \times 10^{10}$ to $3 \times 10^{11}$ $M^{-1}$) were at least two orders of magnitude greater than those corresponding to the published values of $K_D$ (7). Consistent with this higher estimate for $K_{ass}$ is the argument that if $k_f$ and $k_d$ had the values determined experimentally in our study and if $k_r$ had a magnitude consistent with the literature values of $K_D$, the complex should readily dissociate with time. This effect was not observed experimentally. Previous studies (7) allude to the "stickiness" and irreversibility of the [$^3$H]-2,3,7,8-TCDD-Ah receptor protein complex compared with reversible dissociation of some steroid receptor-hormone complexes; the revised equilibrium constants determined in this study more accurately reflect the unique properties of the 2,3,7,8-TCDD-Ah receptor complex and are also consistent with the unusually high potency and persistence of the *in vivo* effects of 2,3,7,8-TCDD.

The activation parameters for the Ah receptor protein-2,3,7,8-TCDD complexation reaction and for the inactivation of the uncomplexed receptor protein were calculated from the temperature dependence of $k_f$ and $k_d$ over the temperature range 10-37°C. The kinetic data determined at 37°C was suspected because the fast reactions did not permit the collection of a sufficient number of data points. The values of the activation parameters are given in Table 3. The "graphical" data are those obtained from conventional Arrhenius plots (Figure 4); the "computational" results are based on a new one-step methodology which

allows activation parameters to be calculated directly from the raw experimental measurements without having to obtain the rate constants as an intermediate step. The new method allows the uncertainty in the activation enthalpy, expressed as a 95% confidence interval, to be obtained unambiguously. This point will become important below. The activation parameters are consistent with deep seated conformational changes in the protein, attendant upon both complexation and inactivation. The activation parameters for complex formation are entirely in accord with this model of "hydrophobic interaction"; the gross conformational change is reflected in the large positive $\Delta H_f^{\ddagger}$, indicating that the transition state many hydrogen bonds are in the process of reorganization, while the very large positive $\Delta S_f^{\ddagger}$ suggests that the driving force in this hydrophobic binding is solvent reorganization as the molecule changes its conformation. Thus the increase in solvent entropy is sufficient to allow binding to be moderately fast at 20°C (and very fast at 37°C) despite the large enthalpic activation barrier that has to be overcome.

The enthalpies of activation for both the formation and inactivation of the receptor-ligand complex are seen from Table 3 to be the same both graphically and computationally, within the statistical uncertainty. In addition, the fitted values of $[R_o]$ are temperature-dependent (Table 1) even though the protein content of he cytosol was kept constant in each series of incubations. We were therefore led to consider a second kinetic model (Scheme 2) in which the receptor is activated to an intermediate, I, followed by competitive degradation of the unoccupied receptor and formation of the RL complex, both these latter steps being fast compared with the first.

Scheme 2




Many variants of Scheme 2 are possible; however, the simplest model, which will be discussed here, has none of the steps considered reversible and has $k_o$ representing the rate limiting step. The time-dependence for RL formation is then given by Equation 3 which is a simple exponential function.

$$[RL] = [R_o] \left(\frac{k_F[L]}{k_F[L]+k_D}\right) (1-e^{-k_o t}) \qquad (3)$$

The factor $k_F[L]/k_F[L]+k_D)$ is the fraction of intermediates I that are trapped by the ligand, and can be designated by $\alpha$.

The experimentally measured rate of receptor degradation in the absence of 2,3,7,8-TCDD leads to the rate constant $k_o$ according to this model. The initial rate of complex formation is given by Equation 4.

$$\frac{\Delta [RL]}{\Delta t} = k_o[R_o]\alpha \qquad (4)$$

At a single temperature and in the absence of ligand, $k_d$ of Scheme 1 is associated with $k_o$ of Scheme 2. In order to compare $k_f$ of Scheme 1 with an appropriate parameter in Scheme 2 we have, from the initial rate experiments, $k_f[L] = k_o\alpha$.

Scheme 2 provides a ready explanation of why the enthalpies of activation for complex formation and receptor degradation should be the same: both depend on the temperature coefficient of $k_o$ only. The entropies of activation are different due to the multiplying term $[R_o]$ in the case of complex formation.

The conclusion that ligand binding and receptor degradation both involve the protein in a conformational reorganization remains unchanged.

It was noted above that $k_f[L] = k_o$ , where $k_o$ is identified with $k_d$. The values of $k_f[L]$ are given in Table 1 and when [TCDD] = $10^{-9}$ M, a value of $\alpha$ in the range of 0.58-0.75 will accommodate the kinetic data over the temperature interval studied. Thus, provided that Scheme 2 is operative, this concentration of 2,3,7,8-TCDD will trap roughly two thirds of all the activated receptor molecules. Since only a fraction of the initial receptor concentration ($R_o$) becomes ligand bound, then the model represented by Scheme 2 also indicates that $R_o$ is underestimated using the traditional Scatchard analysis.

Figure 5 shows a fit of the experimental saturation binding data to Scheme 2 at 20°C, using $[R_o] = 1.22 \times 10^{-10}$ mol $L^{-1}$, $k_o = 0.033$ $min^{-1}$, and $\alpha = 0.75$. The fit of the experimental data to both Schemes 1 and 2 (Figures 3 and 6) was comparable. Analogous stimulations at 10°C and 30°C were also similar in appearance. We consistently observed that the experimental points at intermediate stages of saturation lay below the stimulated curve. This suggests that the true kinetic behavior of the 2,3,7,8-TCDD-Ah receptor protein interaction may be more complex than predicted by either of our Schemes. One possibility might be a two stage binding process (22), but we have not attempted to model this possibility because of the difficulty of assigning physical meaning to the derived rate constants as the complexity of the kinetic model and the number of fitted parameters increases.

In conclusion, we have shown that a simple association model does not account for the binding of TCDD to the Ah receptor. Conclusions obtained by a Scatchard analysis of experimental ligand association data underestimate both $K_{ass}$ and the total concentration of receptor sites. The binding of 2,3,7,8-TCDD

to the Ah receptor is thus qualitatively and quantitatively different from the behavior of steroid hormone receptors. First, receptor degradation occurs competitively with binding over the whole range of temperatures from 4 to 37°C; second, there is as yet no direct evidence for complex dissociation, and as a result, third, binding of the ligand stabilizes the receptor to an extent much greater than is found with steroid hormone receptors.

We have advanced two different kinetic models to rationalize our experimental data. In Scheme 1, association is viewed as being in competition with receptor degradation. According to this model, previous estimates of the association constant $K_{ass}$ are orders of magnitude too low (if reversal occurs at all), and the initial cytosolic receptor concentration has been underestimated by a factor of 2-3. In Scheme 2, we postulate the slow conversion of the Ah receptor to an activated state, which either inactivates or is competitively trapped by 2,3,7,8-TCDD. In the simplest version of this model, none of the steps are considered as reversible but the model can readily be extended to accommodate reversibility. The introduction of Scheme 2 is based principally on the coincidence of $\Delta H_f^{=}$ and $\Delta H_d^{=}$ and represents a novel mechanism of receptor-ligand interaction, namely the activation of the receptor prior to binding. However, before this mechanism can be seriously considered, it will be imperative to show that the same coincidence of $\Delta H_f^{\ddagger}$ and $\Delta H_d^{\ddagger}$ is found for a whole series of halogenated aryl hydrocarbon radioligands, rather than just one. Current studies in our laboratory, utilizing new radioligands and cytosolic receptor protein from different animals species, are in progress, in order to provide a better understanding of the interaction between the Ah receptor and members of the PCDD and PCDF families.

2. Photoaffinity Labeling Studies

Photoaffinity labeling has been carried out successfully on a large number of receptor systems including those for the steroid hormones. Attempts to photoaffinity label (PAL), the Ah receptor(s) have been complicated by the low concentration of this protein in most tissues (i.e., hepatic < 100 fmol/mg) which is a problem not encountered with many other receptors. However, Poland et al., (17-19) have recently reported the photoaffinity labeling the Ah receptor utilizing an $^{125}I$ radioligand which contained a photoreactive azide group. Research on the photochemistry of heterocyclic chemicals indicate that the PCDDs, should be susceptible to structural change under the appropriate photolytic conditions. Most studies on the photolytic degradation of 2,3,7,8-TCDD and structurally similar chemicals have been carried out in organic solvents such as ethanol and acetonitrile. As a result, the photolysis of 2,3,7,8-TCDD in $H_2O$ was carried out and the results indicated that 2,3,7,8-TCDD is rapidly photolyzed to unknown product(s) when irradiated with ultraviolet (UV-A, 250-400 nm) light. The observed loss of 70% of the starting material within the first 15 min of photolysis indicates that this ligand is significantly photolabile within the time frame of stability of the liganded Ah receptor in the cytosolic preparations.

The stability of the Ah receptor under two defined sets of photolysis conditions; one involving UV-A radiation ( ~ 250-300 nm), a second involving UV-B radiation ( > 300 nm), were determined. The results show (Figure 6), that with UV-A irradiation being of significantly higher energy, loss of unbound receptor is more rapid than with the lower energy source UV-B. Nevertheless, with the higher energy light source, we observe a relatively small loss of receptor binding activity within the time frame required for photolytic loss of

2,3,7,8-TCDD.

When the [$^3$H]-TCDD receptor complex is photolyzed potocovalent adduction of the radioligand to proteins in the cytosol was observed. Figure 7 shows preliminary evidence of photoinduced changes in the form of increased binding to a number of components (varied molecular weights separated with sucrose density centrifugation. Precipitation of the photolyzed cytosolic protein with acetone (which solvates and removes excess unbound ligand) provides direct evidence for photocovalent attachment of the radioligand. In the absence of photolysis (time 0), acetone treatment removes all radioactivity from the precipitated protein pellet, whereas, with increasing photolysis time, an increase in unextractable radioactivity was observed in the protein pellet.

With evidence that the radioligand, [$^3$H]-2,3,7,8-TCDD was covalently adducted to cytosolic protein, the proteins were separated with sodium dodecycl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE). As expected with a crude preparation such as hepatic cytosol, the coommassie-stained polyacrylamide gel illustrated the complex mixture of proteins which were present. The gel was impregnated with a fluorgraphic enhancer, dried and loaded on ultra-sensitive X-ray film and stored at -70$^o$C for exposure. Development of X-ray film(s) after appropriate exposure time showed little or no detectable exposure pattern. A detectable pattern was observed only after film exposure of the gel for a minimum of 24 weeks, however very little significant information could be obtained. The lack of specificity of the photolabeling/autoradiography was due to (a) the properties of tritium and a weak emitter and (b) the Ah receptor content of the crude cytosol preparation was too low. Thus autoradiography was not a convenient and reasonable method for localizing specifically adducted $^3$H-TCDD. This problem was circumvented by utilizing a method involving slicing the

polyacrylamide gel lanes into 2-3 mm slices and determining the radioactivity content of each slice. This was accomplished using a Biological Oxidizer which oxidizes the constituents of the gel slice at 800°C to $H_2O$ and $CO_2$ followed by condensation of the $H_2O$ into an emulsion with scintillation cocktail. The radioactivity contained in the gel slices was thus determined by liquid scintillation counting of the recovered tritiated water (> 95% recovery).

A typical gel profile is shown in Figure 8 and specific labelling of a 95, 90 and 71 kDa protein subunits were observed. This was in good agreement with Poland results reported by and coworkers (17-19) where specific labelling of a 95 kDa fragment were observed. Poland et al. (19) also reported that the 70 kDA labelled subunit is a proteolytic fragment of the 95 kDa protein which is now thought to be a true monomer of the functional Ah receptor.

3. Purification of the Ah Receptor

Attempts to purify this receptor in its unbound form have been carried out with a variety of biochemical techniques including column chromatography and sucrose density gradient centrifugation. It appeared that biochemical manipulation of the unliganded receptor resulted in a rapid loss of specific ligand binding activity. However, significant stabilization of the receptor occurs when a ligand (ie., 2,3,7,8-TCDD) is bound. When [$^3$H]-TCDD liganded receptor from Long Evan rat hepatic cytosol was separated on an equilibrated Sephacryl-300 column, we observed two specifically-bound radioactive peaks and this procedure resulted in up to a 10-fold purification of the receptor protein. A comparable 10-fold purification was obtained by centrifugation on a 5-25% sucrose density gradient. Current studies have been initiated to utilize a series of column chromatographic and velocity sedimentation procedures to further purify the Ah receptor.

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## FIGURE CAPTIONS

Figure 1. Time course for specific binding of 1 nM [$^3$H]-2,3,7,8-TCDD to the Ah receptor at 10°C (■), 20°C (●) and 30°C (▲).

Figure 2. Time course of receptor degradation as judged by binding [$^3$H]-2,3,7,8-TCDD to the receptor after pre-incubation of cytosol in the absence of ligand for the times indicated.

Figure 3. Fit of the observed experimental binding of [$^3$H]-2,3,7,8-TCDD to the Ah receptor at 20°C ( ) to the computer stimulated binding curve (A) according to Scheme 1. Curve B is a simulation of the time course of binding of [$^3$H]-2,3,7,8-TCDD-Ah receptor at 20°C using values of $R_o$, $k_f$ and $k_d$ from Table 1, but with $k_r$ chosen to give $K_D$ = 2.5 nM.

Figure 4. (a) Arrhenius plot (ln $k_d$ vs. 1/T) for degradation of the unoccupied Ah receptor.

(b) Arrhenius plot (ln $k_f$ vs. 1/T) for complexation of [$^3$H]-2,3,7,8-TCDD to the Ah receptor.

Figure 5. Fit of the experimental binding of [$^3$H]-2,3,7,8-TCDD to the Ah receptor at 20°C ( ) to the computer simulated binding curve (——) according to Scheme 2.

Figure 6. Effects of U.V. wavelength on photolytic inactivation of the Ah receptor.

Figure 7. Photocovalent adduction of [$^3$H]-2,3,7,8-TCDD to rat hepatic cytosolic receptor.

Figure 8. SDS polyacrylamide gel slices of rat hepatic cytosol photolyzed in the presence of [$^3$H]-2,3,7,8-TCDD (control) and [$^3$H]-2,3,7,8-TCDD plus a 20-fold excess of 2,3,7,8-TCDF (competed).

Fig 1

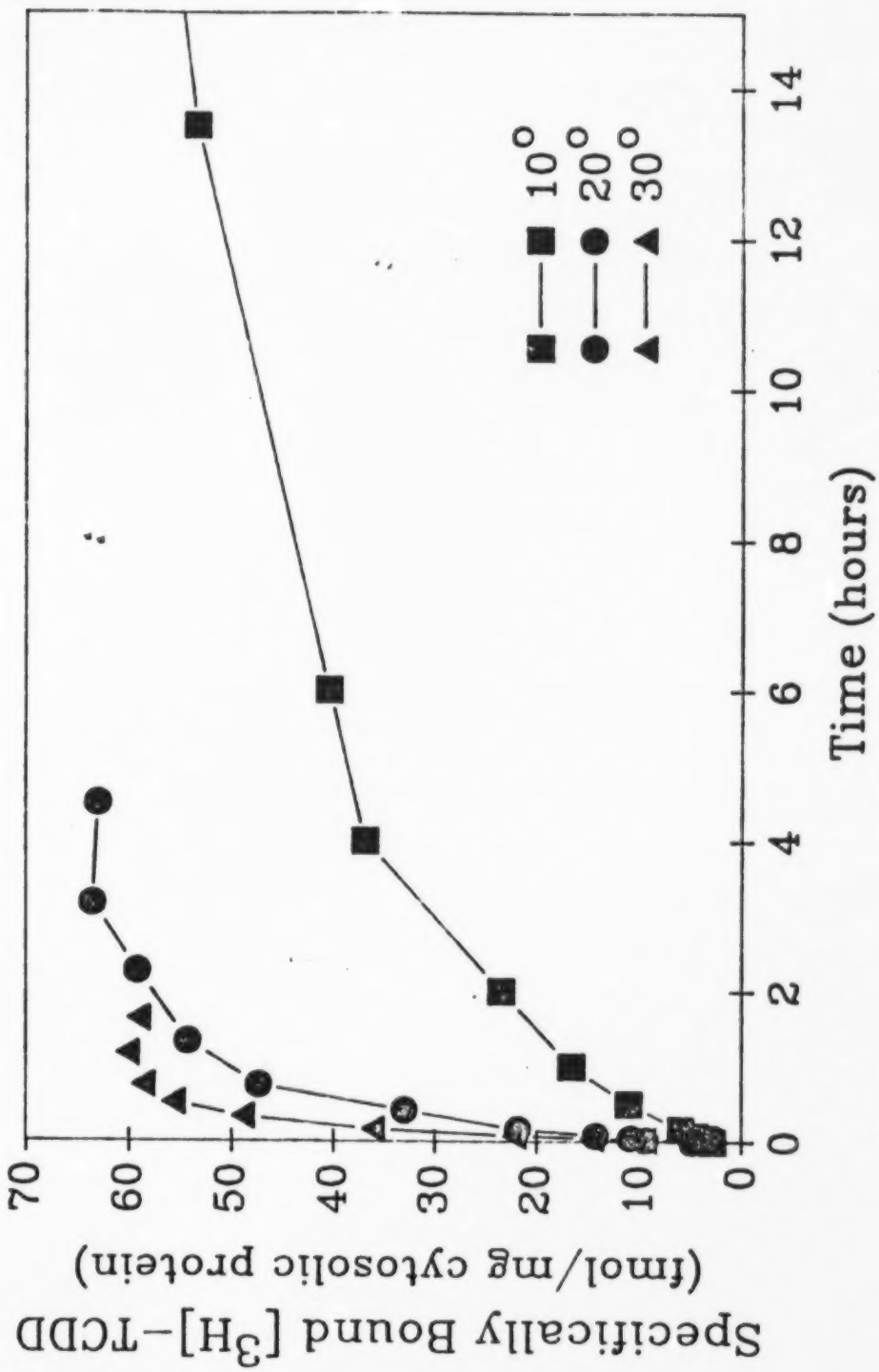

Specifically Bound [3H]–TCDD
(fmol/mg cytosolic protein)
Time (hours)
10°
20°
30°
0
10
20
30
40
50
60
70
0
2
4
6
8
10
12
14

Fig 2

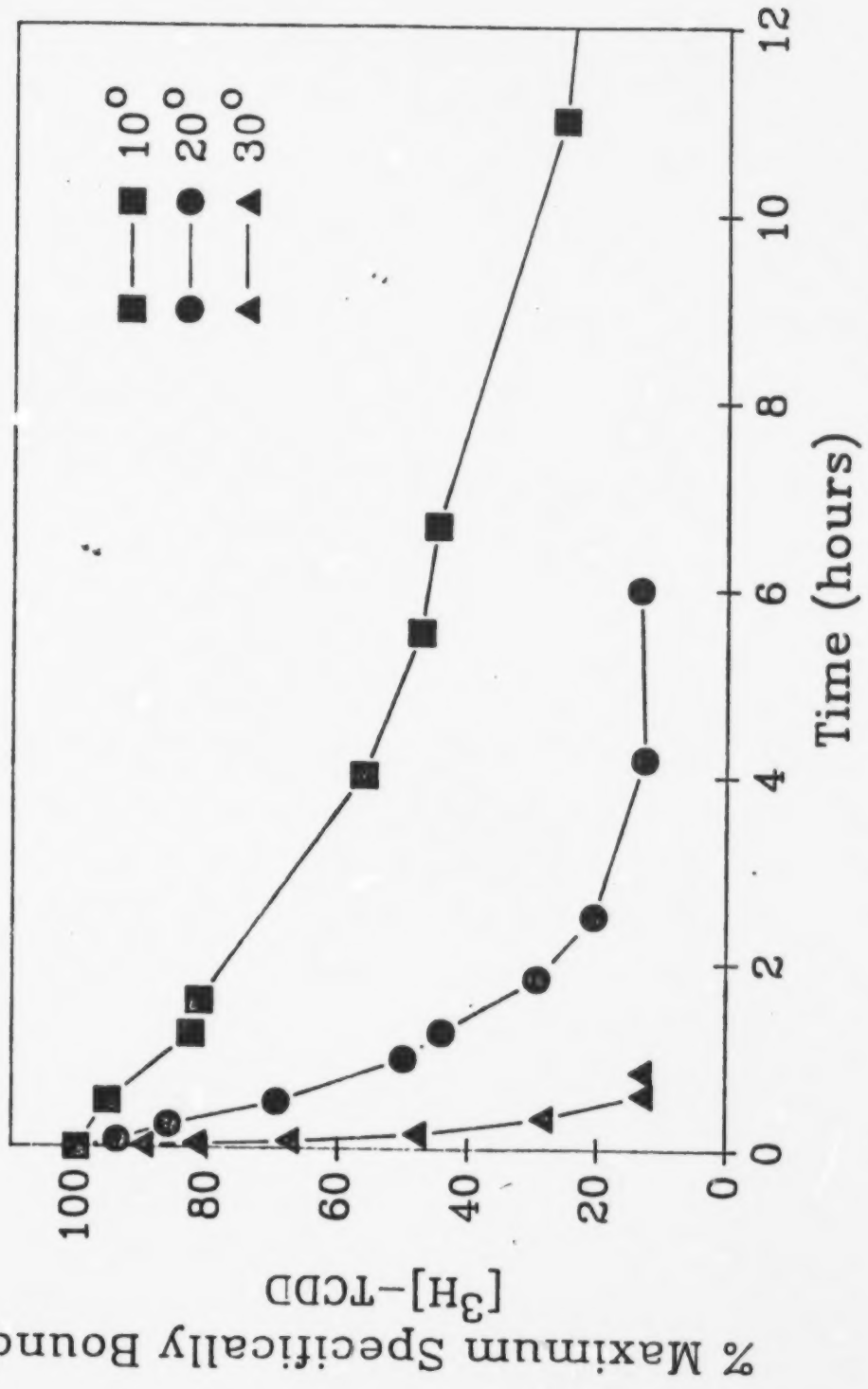

% Maximum Specifically Bound
[3H]-TCDD
100
80
60
40
20
0
0
2
4
6
8
10
12
Time (hours)
10°
20°
30°

Fig 3

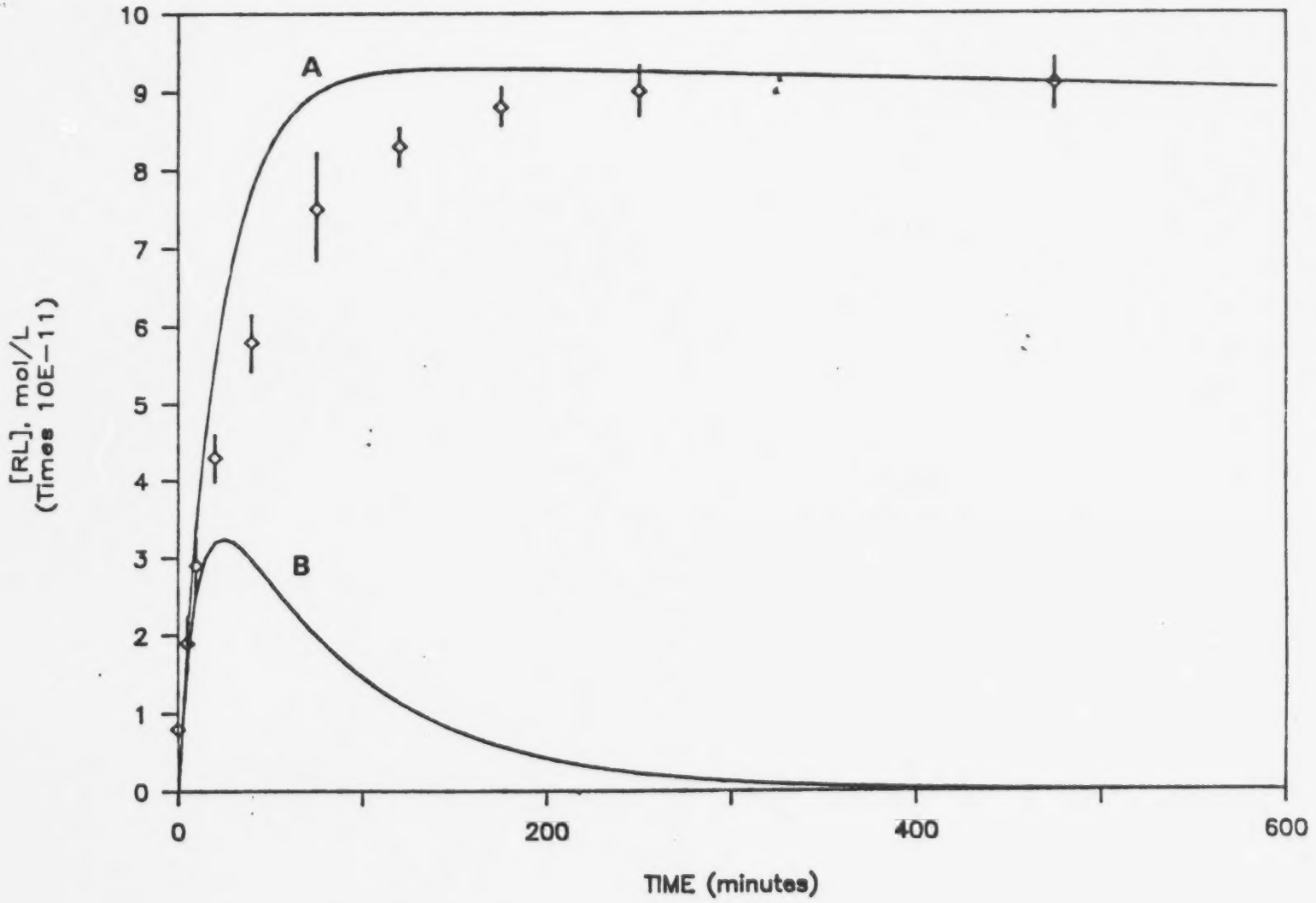

10
9
8
7
6
5
4
3
2
1
0
A
B
[RL], mol/L
(Times 10E-11)
0
200
400
600
TIME (minutes)

Fig 4a

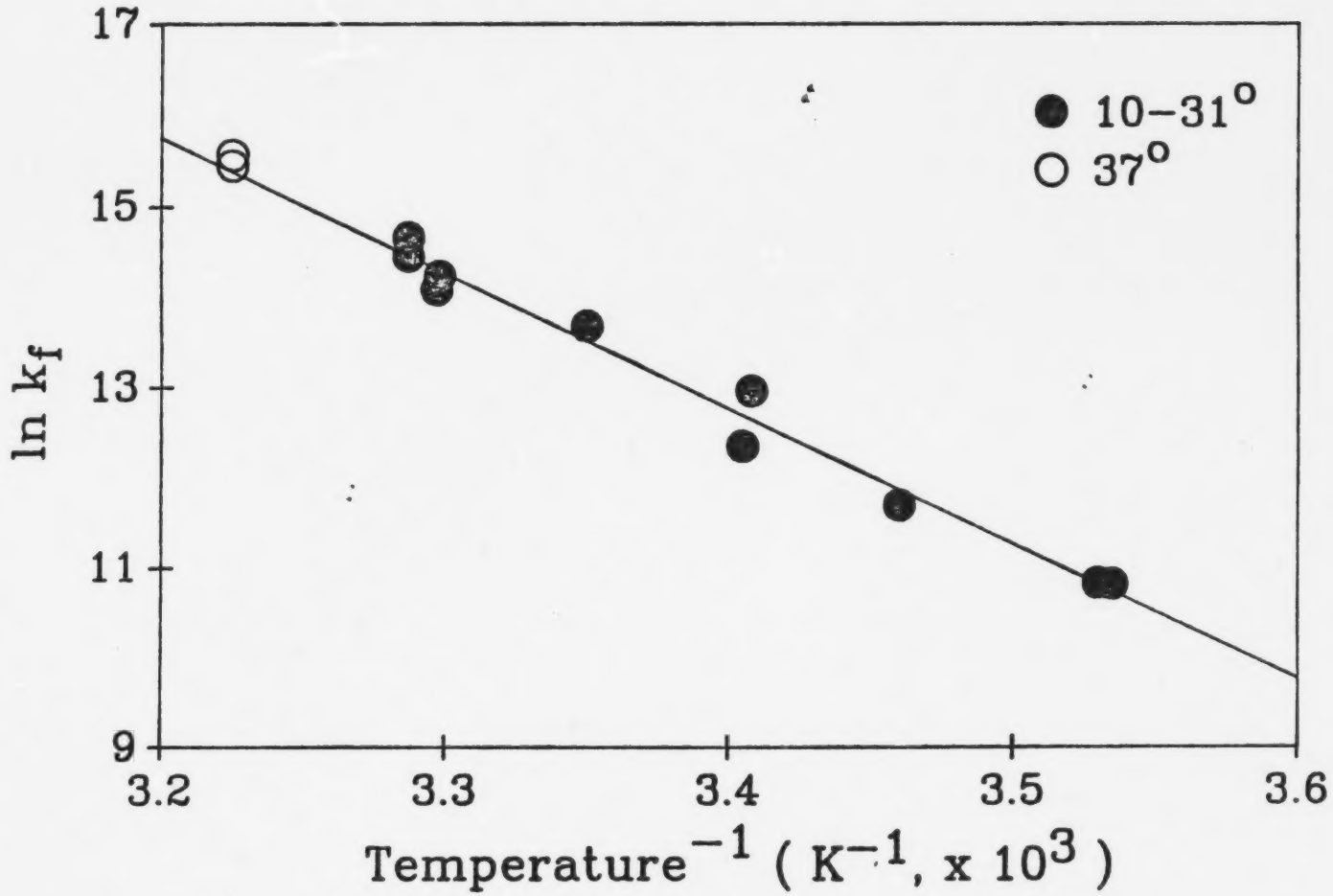
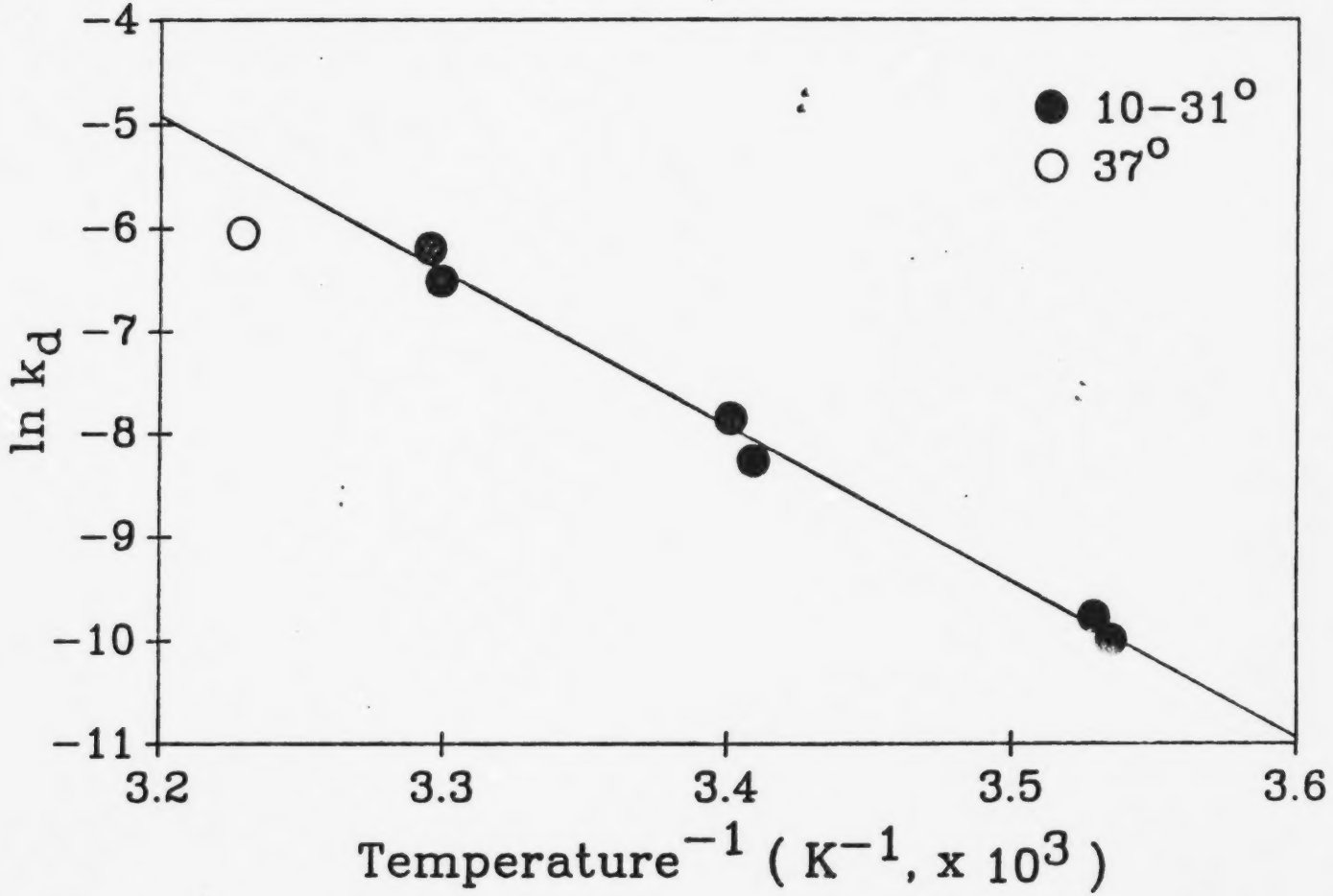


fig 4b



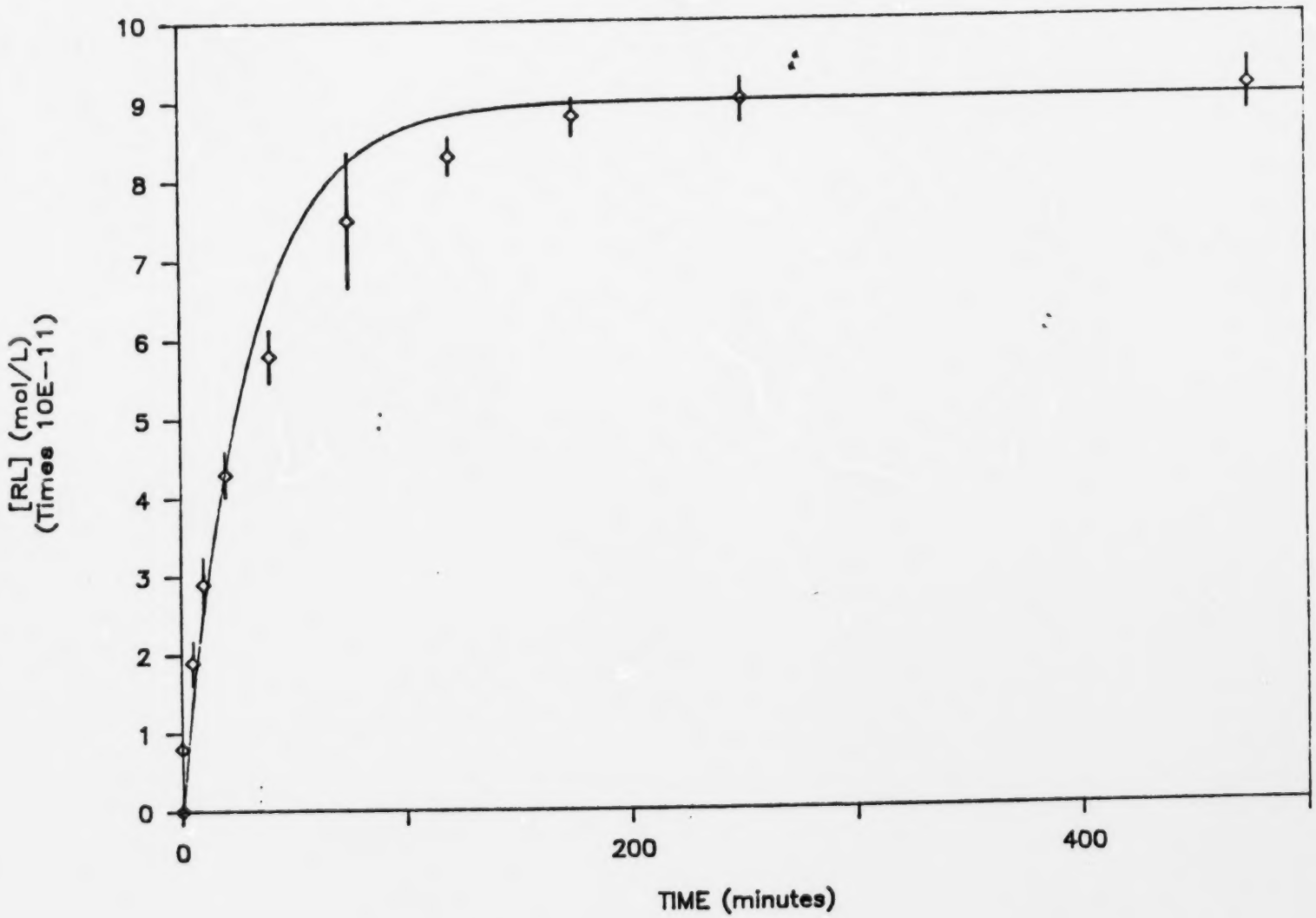

10
9
8
7
6
5
4
3
2
1
0
[RL] (mol/L)
(Times 10E−11)
0
200
400
TIME (minutes)

Fig 6

# EFFECTS OF UV WAVELENGTH ON PHOTOLYTIC INACTIVATION OF THE Ah RECEPTOR

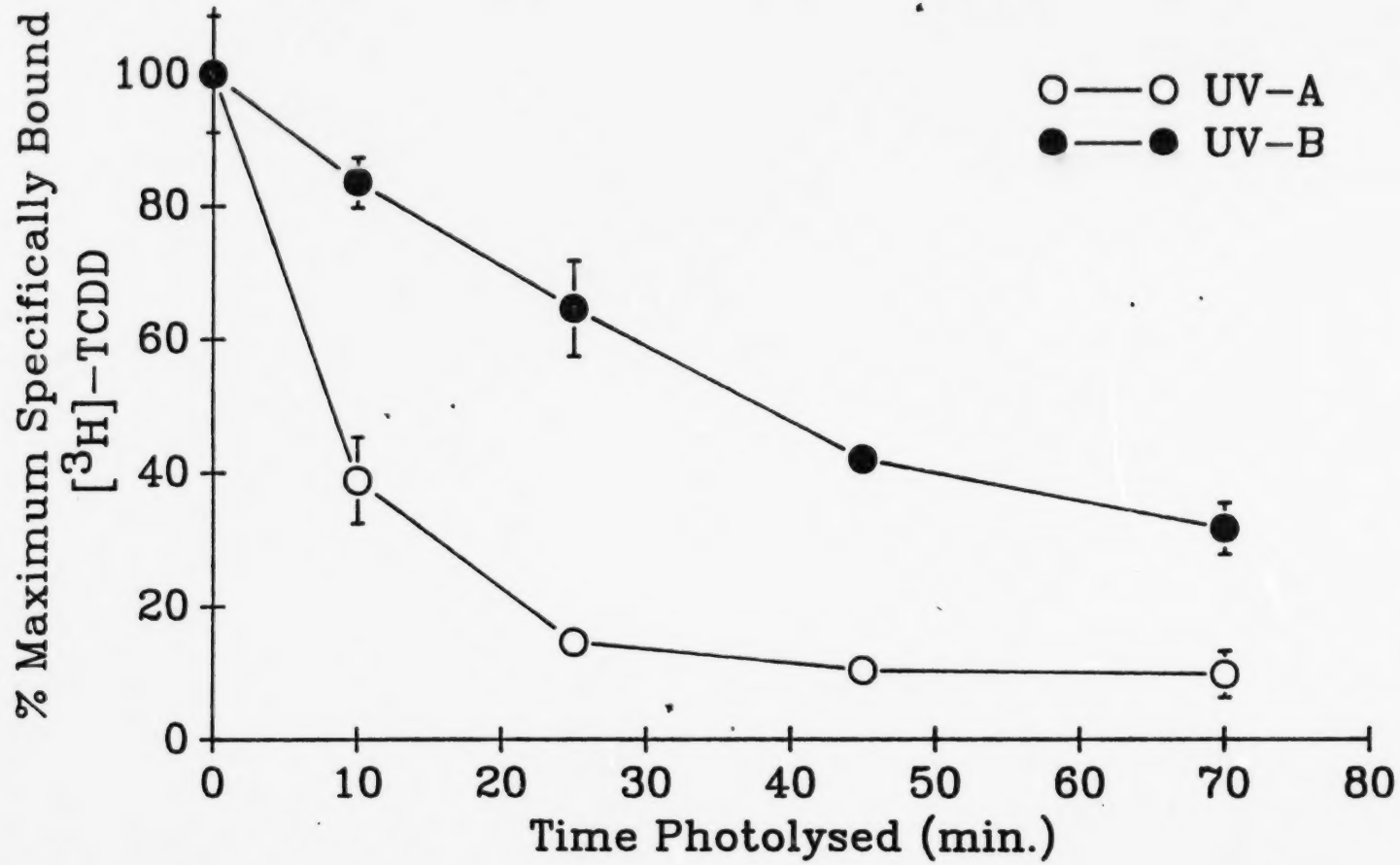


Fig 7

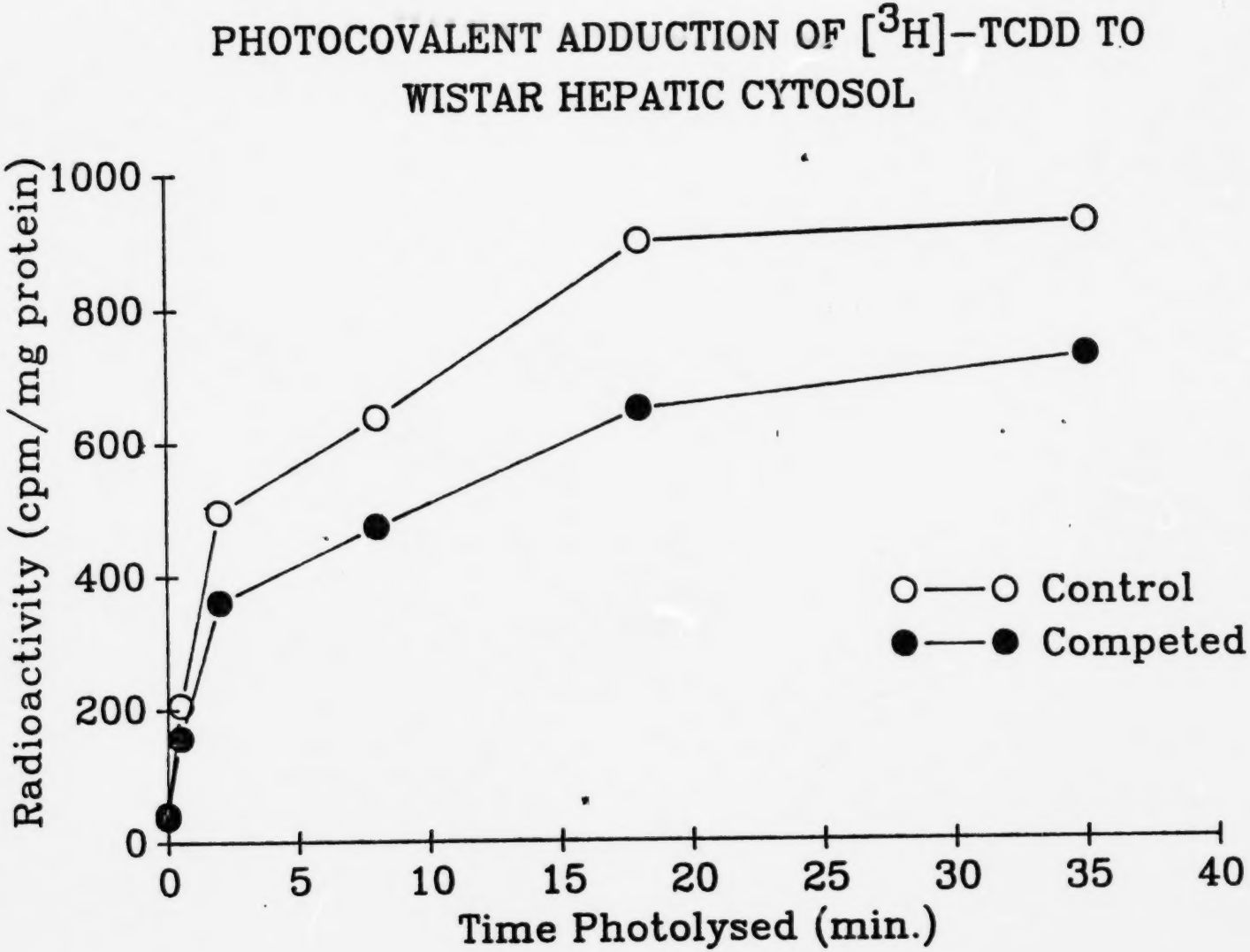
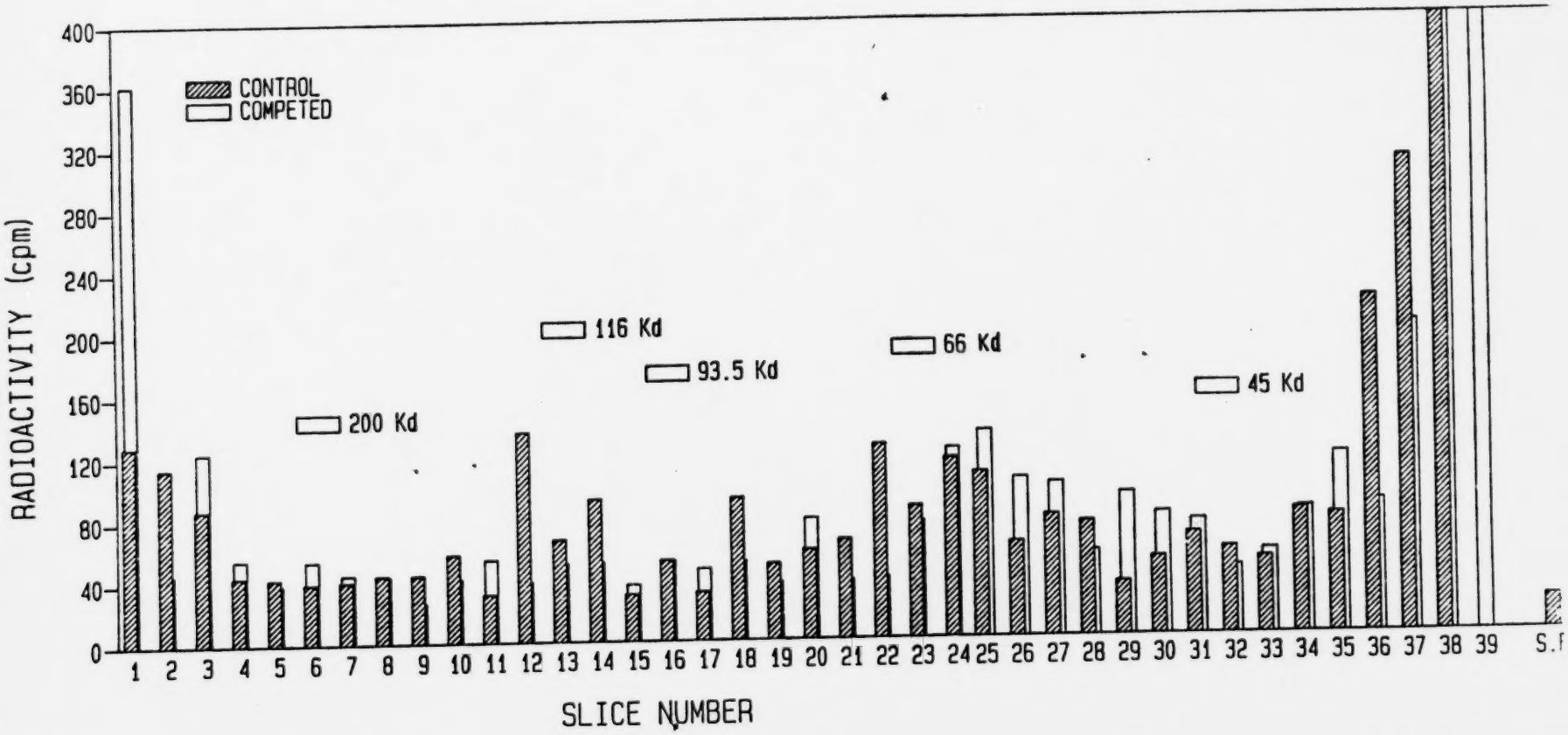

PHOTOCOVALENT ADDUCTION OF [3H]–TCDD TO WISTAR HEPATIC CYTOSOL
Radioactivity (cpm/mg protein)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
Time Photolysed (min.)
Control
Competed

Fig. 8


CONTROL
COMPETED
200 Kd
116 Kd
93.5 Kd
66 Kd
45 Kd
RADIOACTIVITY (cpm)
SLICE NUMBER
0
40
80
120
160
200
240
280
320
360
400
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 S.F